II - First Acid Fraction

S S S 56 B & C Hydrolysis

DEC 4 rff. — II Taken up in H_2O, bone-blacked, but color remained. Concd. to sm. bulk in vacuo, working up w. alc. & abs. alc. until friable & could be scraped from flask. Dried in vac. Yield: 4.4 g.

Wt. bot.(8) + subst., dried in vac. in $CHCl_3$ oven 8.2400 8.2368 8.2353

7.7055

.5219 Ash-free. .5298

.0079

Made up to 10 cc.

1.5% Ash ~~0.8%~~

	ASH		
Wt. ~~bot~~ + subst	7.7055	Wt. crux + ash	6.7775
" "	7.5944	" "	6.7747
	.1111	" $BaSO_4$	.0028 .58837
		" Ba	.00165 ~~.24435~~

Ba from poor basic PbHC

DEC 22 — Optical ROTATION

$l = 1$ Bl. −0.06 .06 .06 Rdg. +0.26 .26

$$[\alpha]_D = \frac{32}{.5219} \times \frac{10}{100} = +6.1^\circ$$

ACID EQUIVALENT

1 cc. sample. Phenolphthalein. cc. N/50 NaOH (81.049)

5.98

5.70 = .119 cc. N NaOH

Endpt. quickly faded, indicating opening up of lactone. This was repeated as alk. was added a few drops at a time, until burette read 8.6 cc. = 9.02 = 0.180 cc. N

Acid Equiv. ~~427~~ 438

292

DEC 24 Repetition ~~Macro~~

8.3 cc. = 8.7 = .174 cc. N. 302

Macro Schaffer Hartmann (Goebel) 2 cc. sample

Bl. 50.0 N/10 $Na_2S_2O_3$ 50.00

Rdg 34.0 " " (81.015) 34.5

$$\frac{15.5 \times 6.36}{2.18} \times \frac{100}{.1051}$$

Redg. Sugars 43%

	MICRO	1 cc. dil. to 25	2 cc. samples
JAN 5 1927 — Bl. 19.05	19.15	19.10	
		7.95	
Tity. 7.9	8.0	11.15 × 1.012 × .318	

= 3.59 mg. Cu = 1.66 mg. glucose

Redg Sugars 39.5%

In 25 cc. .0522 g.

" 2 cc. .0042 g.

5 S S 56 B+C

Repetition of micro-Schaffer-Hartmann on 1.5 cc. samples.

Bl. 19.10 19.10
Rdg. 11.05 10.95 11.00
8.1 × 1.012 × .318 = 2.61 mg. Cu
= 1.26 mg. glucose

In 1.5 cc. .00313 g.

Redg Sugars 40%

Jan. — Converted into Ca salt by Goebel, 1" fract. of this (1.5 g.) decompd. w. sl. < theory of $H_2C_2O_4$ (detd. by anal. of desicc.-dried portion), filtered from CaC_2O_4 w. aid of bone-black, concd. in vac. almost dry, taken up in MeOH, centfgd. from remaining traces Ca salt, concd. dry in vac. Yield: 1.0 g.

Wt. bot (E) + subst, in vac at t. b. $CHCl_3$ over H_2SO_4 7.5262
7.5262 7.5238, over night 7.5223
Diss. in H_2O, made up to 5 cc. 7.3043
OPTICALLY INACTIVE .2180
.0004
.2176

Ash 0.2%

ASH

Wt. bot + subst 7.3043
" " 7.2478
.0565

Wt. dish + ash 17.1684
" " 17.1681
" " + $CaSO_4$ .0003
" Ca .0001

.02176
.00218
.00072

0.5 cc. made up to 20 cc. 2 cc. samples. (Goebel)

18.80
14.75 14.75
4.05 × 1.015 × .318 = 1.32 mg. Cu = .72 mg. glucose

Redg. Sug. 33%

Acid Equivalent.

1 cc. req. 5.1 cc. N/50 NaOH (×1.049) for initial color to phthl. Endpt. not permanent, few drops added at time. Fairly permnt. endpt. 7.2 cc.
7.55 1.51 cc. N .0435 g.

Acid Equiv 288

Willstätter-Schudel

2 cc. orig. soln.
N/10 I. = 10.00 N/20 $Na_2S_2O_3$ (× 0.9876) = 11.25
5.56 11.11
I used up 4.44
10 : 4.44 = 90 : x 40 mg. glucose
Wt. sample 2/5 × .2176 = .087 g.

Aldoses 46%

III – 2" Acid. Fraction.

SSS 56 B+C Hydrolysis

DEC 7 rffl. — III soln. concd. dry several times in vac. & at vol. of ca. 35 cc., reppfld. w. basic PbOAc. Ppt. (III) & supnt (IIIa) wkd. up separately. III finally dried out to friable mass w. aid of abs alc. Detached off, dried. Yield: 2.4 g.

IIIa concd. to symp in vac, dried to friable mass w. Gerle pump, ground up in agate mortar & portion dried const. in vac. at t-b. $CHCl_3$ in bot (A). 9.0995 9.0964 9.0945 9.0904 9.0893

Yield: 3.6 g.

3A DEC 31

Wt. bot.	8.5606
" subst	.5287
	.0804 – Ash
~~Diss. in tac. H_2O.~~	.4483 – Ash-free subst.

Ash 15.2%

ASH

Wt. bot + subst	8.5606	Wt. crus + ash	6.2753	2749 ✓
" bot	8.4272	" "	6.2405	
	.1334	" $BaSO_4$	.0348	5884
		" Ba	.0203	

Ba from poor basic PbOAc

Concd. aq. soln. gives ppt. w. H_2SO_4, not HCl, indicating ~~Ba or Ca~~.

JAN 4 1927 – Sample diss. in H_2O, made up to 10 cc., centfgd. from dust.

OPTICAL ROTATION

$l = 1$ Bl. = -0.07 -.07 Rdgs.: +0.18 .18

$$[\alpha]_D = +\frac{25}{.4483} \times \frac{10}{100} = +5.6°$$

MICRO – Schaffer-Hartmann 1 cc. dil. to 25 2 cc. samples

Bl.			19.10
Rdgs.	7.10	7.25	7.20

11.90 × 1.012 × .318 = 3.83 mg. Cu = ca 1.8 mg. glucose 46%

25 cc. ∝ .0448 g.
2 cc. ∝ .0039 g.
1.5 cc ∝ .0026 g.

Bl. 19.10
Rdgs. 10.55 10.55

1.5 cc. samples

8.55 × 1.012 × .318 = 2.75 mg. Cu = 1.31 mg. glucose

Rdg. sugars 48.7%

Hydrolysis

SSS 56 III

DEC 17 — Wt. bot (1) + subst ($CHCl_3$ oven) 7.7613 7.7580 7.7562 7.7544

Sm. amt. lost. → 7.7533

7.2409

.5124

.0236

.4888 — Ash-free

.5011 — Insol. impur.

.5001

Diss. in H_2O, made up to 10 cc.

DEC 21

ASH

Wt. bot. + subst 7.2409

" " 7.1560

.0849

Wt. crux + ash 6.7814

" " 6.7748

" ($BaSO_4$ .0066

" Ba .0039 .5884

Ba from poor PbOAc

Ash 4.6%

OPTICAL ROTATION

$l = 1$ Bl. = − 0.04 .05 .04 ✓ Rdgs. −0.01 +0.01 −0.01

$$[\alpha]_D = \frac{4}{.4888} \times \frac{10}{100} = +0.8^\circ$$

Reducing Sugars

Macro Schaffer Hartmann (Goebel). 2cc. sample

Bl 50.0 N/10 $Na_2S_2O_3$ 50.00

32.0 " " (×1.015) 32.48

~~32.48 × 0.636~~ ~~.217 × 100/.1 =~~

17.52 × .636 × 100/.1 × .5/.4888 = ~~51.8~~ %.

52.3%

WILLSTÄTTER - SCHUDEL

Bl 20.0 (×.985) N/10 19.7

26.0 N/20 13.0

6.7

6.7/11.11 × 100 = 60.3%.

S S S 56 D

OCT 18 1926 — 200 g. same lot G.A. diss. in 1 l. chilled 1:1 HCl, let stand 2 days. OCT 20 — Centfgd; soln. ppd. w. 2400 cc. chilled alc. After several hrs. decanted ppt. treated w. a 500 cc. alc. OCT 21 — Centfgd. Fractionated same as preceding 56, fractions combined, washed thoroughly w. acetone, dried in vac.

A: 34.0 g.
B: 6. g.
C: 52.8 g.
92.8 g.

S S S 56 A

NOV 2 - Wt. bot (3) + subst 8.5076 8.4869 8.4829 8.4804 8.4789 8.4769
8.4752 8.4748

Diss. in H_2O, made up to 25 cc.

7.5794
.8954
.0010
ASH .8944

Wt. bot + subst	7.5794	Wt. dish + ash	17.1709
" "	7.4107	" dish	17.1703
	.1687	" $CaSO_4$	.0006
		" Ca	.00018

Ash 0.11%

OPTICAL ROTATION

$l = 2$ Bl. = −0.04 .03 .04 Rdgs. −0.88 .85 .86

$$[\alpha]_D = \frac{82}{2 \times .8944 \times 4} = -11.5^\circ$$

$[\alpha]_D$ Acid −11.5°

ACID EQUIVALENT

15 cc. soln. 0.02 cc. phenol red 8.1 cc. N/14 NaOH (x1.050)
.5368 g. ∝ 8.51 cc. N/14 ∝ 0.608 cc. N

Acid Equiv. 883

MICROKJELDAHLS 1 cc.

cc. N/70 HCl (x.986)	10.00	9.86	10.00
" " NaOH (x.9,96)	9.46	9.43	9.47
		.43	
		.1	
		.33 × .2 = 0.066 mg N.	

1 cc. = 0.0358 g.
35.8 mg.

N 0.18%

SSS 56 B

NOV 2 - Wt. bot ( ) + subst 9.2750 9.2735 9.2721 9.2716 9.2717

8.4507

Diss. in H_2O, made up to 25 cc. .8210

Less Ash .0006

.8204

ASH

Wt. bot. + sample 8.4507

" " 8.3146

.1361

Wt. bot. + ash 17.1702

" " 17.1699

Wt. Ash .0003

" Ca .00009 factor 0.29435

% Ash
0.07

NOV 8 AON

OPTICAL ROTATION

$l = 2$ Bl. = -0.02 .02 Rdgs. -0.55 .55 .54

$$[\alpha]_D = -\frac{53}{2 \times .8204 \times 4} = -8.1°$$

$[\alpha]_D$ Acid
-8.1°

Acid Equivalent

15 cc. soln. 0.05 cc. indicator (phenolred) 7.98 cc. N/14 NaOH (×1.050)

8.38 cc = 0.598 cc. N NaOH

.492 mg. of 0.598 cc.

Acid Equiv.
823

MICROKJELDAHLS

1 cc. samples

cc. N/10 HCl (×0.986)	10.00		10.00
" " NaOH (×0.996)	9.61	9.86	9.68
		9.61	
		.25	
		.1	
		.15 ×0.2 = 0.03 mg. N	

1 cc = 0.0328 g

32.8 mg.

N
0.09 %

SSS 56 C

NOV 2 - Wt. bot (8) + subst 8.6216 8.6183 8.6160 8.6150 8.6144

7.5475

Diss. in H_2O, made up to 30 cc. 1.0675

Ca .0002

ASH 1.0673

Wt. bot + subst	7.5475	Wt. dish + ash	17.1703
" "	7.3880	" dish	17.1702
	.1595	Wt. $CaSO_4$	.0001
		" Ca	.00003

Ash
0.02%

NOV 8

OPTICAL ROTATION

$l = 2$ Bl. = −0.02 .03 .03 Rdgs. −0.68 .69 .67

$$[\alpha]_D = -\frac{65 \times 30}{2 \times 1.0673 \times 100} = -9.1°$$

$[\alpha]_D$ Acid
−9.1°

Acid Equivalent

~~1~~5 cc. soln. 0.02 cc. phenol red. 8.16 cc. N/14 NaOH (×1.050).
0.5337 g. req. 8.57 cc. N/14 = 0.612 cc. N.

Acid Equiv.
872

OPTICAL ROTATION, Na Salt.

$l = 2$ Bl. −0.05 .05 .05 Rdgs. −0.68 .66 .69

$$[\alpha]_D = -\frac{62 \times 36}{2 \times 1.0673 \times 100} \times \frac{23.18}{25} = -13.5°$$

$[\alpha]_D$ Salt
−13.5°

MICROKJELDAHLS 1 cc. samples.

cc. N/20 HCl (×0.986)	10.00	9.86	10.00
" N/70 NaOH (×0.996)	9.64	9.61	9.65
		.25	
		.1	
		.15 × 0.2 = 0.03	

1 cc. = 0.0356 g.
= 35.6 mg.

N
0.08%

S. type I 58

0.3% Glucose in broth

3/22/26	Abbe I Dx	III-12, 15, 16	1, 1, 10	3/23	3/24
3/23	" "	III-16	12	3/24	3/25
3/24	" "	III-16	10	3/25	3/26
3/29	" "	III-23	12	3/31	4/1
3/30	" "	III-23	12		
3/31	" "	III-23	13	4/1	4/2
4/1	" "	III-23	14	4/2	4/3
4/2	" "	III-23	20/105	4/5	4/6
~~4/6~~	~~Syn. Broth~~	~~—~~	~~19~~	~~4/6~~	~~4/8~~

~~Flasks 125~~
~~Liters 187½~~

All of above lots were ppt. with 1.2 alcohol & neutralize with alkali.

Flasks	105
liters	157½

157.5 liters

APR 8 — SSS liquid was about 2700 cc. This was neutralized with NaOH and then ppt. with 1500 cc alcohol. After 2 hrs. tested positive then added 500 cc more of alcohol. Then put in ice-box overnight.

2

APR 9 — A small sample was centrifuged - Tested - positive. 700 cc more of alcohol was added.

APR 10 — Supernatant was sucked off. Then 13 250 cc bottles were filled with bottom layer & centrifuged. Bottom layers (no middle layers) were dissolved in about 1.5 liters of water - centrifuged. Clear liquid with S was poured in battery jar. Then sediment was washed - centrifuged & suprnt. added.

HCl APR 12 — Made acid to Congo w. 1:1 HCl, let stand in cold. APR 13th — Centfgd.,

3 ppt. washed 1" w. 200 cc. N/100 HCl. Suprnt. & washings, at vol. of 1900 cc. pptd. w. 3 l. alc. Let stand in cold overnight, suprnt. decanted, centfgd., ppt.

HOAc washed w. 200 cc. N/2 HOAc, let stand in cold ca. 2 hrs., centfgd. Suprnt., weakly +, colored, discarded. Ppt. taken up in H_2O, neutred w. NaOH, centfgd. At

4 vol. of 500 cc. 20 g. NaOAc added, pptd. w. 300 cc. alc., let stand

over night in cold. APR 17 – Centfgd., taken up in ca. 500 cc. H_2O, centfgd. (warm)
HOAc APR 19 – off. Small ppt. added to next batch., supnt. stirred w. 20 cc. HOAc, let stand
2 hrs. in ice-box. Necessary to ppt. w. alc. Centfgd. Ppt. rediss. w. H_2O & alk., & at vol. of 400 cc., 200 g. NaOH
6 added w. 250 cc. made alk. & pptd. w. alc., but necessary to acidify w. HOAc
before all came down. Centfgd., taken up in H_2O + little NaOH, & at vol. of
6 400 cc., pptd. w. 250 cc. alc. Let stand over night, centfgd. Taken up
HOAc in ca 300 cc. H_2O, pptd. w. 9 cc. HOAc. Next day centfgd., neutrd., taken
up in ca 300 cc. H_2O. Saved for Remainder.

Total
471 liters

OCT 14 1926 — Most of S had apparently pptd., so repeatedly centfgd. until compact deposit obtained. Supnt., –, discarded.
NOV 24 — Ppts. taken up in H_2O, & at total vol. of ca. 800 cc., dissd. by adding 1:1 HCl in very sl. xs. Centfgd., slight ppt. discarded, & still turbid solns. neutrd. back, w. stirring to max. pptn. w. 10% aq. NaOH, as in case of #59. NOV 26 – off. Centfgd. Supnts., –, contg. a little glycogen, discarded. Ppt. rediss. in H_2O + just enough 1:1 HCl, centfgd., again pptd. as before. Supnt., –, still contd. ~~much~~ trace glycogen. DEC 2 & 3 – Rediss. as before, dil'd. to ca. 1 l., centfgd. & opalescent soln. run through acid-washed 8 in Berkefeld V candle, coming through clear. Pptd. by adding 10% NaOH until only very faintly blue to Congo. Let stand in cold, ~~over night~~. DEC 6 – Supnt. only faintly +. Centfgd. & supnt. discarded. DEC 7 – Ppts. rediss. in acid, pptd. as before. Still colored. DEC 8 – off. Centfgd. Ppts. taken up in H_2O + NaOH &
$Ba(OH)_2$ at vol. of ca. 600 cc. pptd. in cold w. hot satd. $Ba(OH)_2$.
Ppt. centfgd. off after ½ hr., supnt., weakly +, discarded. Ppt. diss. in cold in H_2O + xs. HCl, pptd. w. chilled alc. Rediss. in H_2O + little HCl, xs. H_2SO_4 added, centfgd., & washings of ppt. added. S pptd. several times as HCl salt w. alc. to remove most of H_2SO_4, then several isoelectric pptns. from acid side finally 2 x as HCl salt w. redistd. alc. then diss. in a little H_2O, pptd. w. redistd. alc. & finally washed with H_2O, alc., acetone as usual.

Yield: 7.7 g.

S S S 58 Type I

5/4/26	Abbe I	Dx	IV 27	18	5/7	~~137~~	
5/5/26	"	"	" 27, 28	19			
5/6/26	"	"	" 28	14			
5/7/ "	"	"	" "	15	5/8		
5/10 "	"	"	" "	14	5/12		
5/11 "	"	"	" "	18			
5/12 "	"	"	" "	14	5/14		
5/13 "	"	"	" "	18			
5/14 "	"	"	" "	13			
5/18	"	"	V - 6, 11, IV - 28	11, 10, 1/15 ✓		5/19	5/20
5/27	"	"	V - 21	11		5/28	5/29
dense 5/28	"	"	V 15, 19, 20, 21	1, 1, 2, 11		6/1	6/2
6/2	"	"	V - 25	15		6/3	6/4
6/3	"	"	V - 25	16		6/4	
~~6/5~~	~~"~~	~~"~~	~~V - 25~~	~~16~~	6/9		

Flasks 209 ✓
Liters 313.5

313,5 P.

The first nine batches of the above were ppt. with 1.2 alc, But the rest was ppt with 1.3 alc. excepted one marked dense which was ppt. with 1.4 alcohol.

JUN 8 The whole of S.S. solution was 11.7 lbs after dissolving crystals and rinsing. This was ppt. with 5 Ls of alcohol and then set in ice-box ~~4 hours~~ for several hours. Tested - positive. Added one more liter of alcohol and set in ice-box overnight. Tested - negative. Centrifuged bottom layer in 16 bottles. Dissolved bottom layer in water (no middle layer) Centrifuged.

JUN 9 Total S.S. solution 3000 cc. This was ppt. with about 500 cc. of 1:1 HCl. and Then set in ice-box over lunch hour. Centrifuged. Poured clear S. solution in 14 Ls jar and washed sediment twice.

HCl JUN 10 Total S solution 3½ liters
" Alcohol 7 " left overnight in ice-box.

JUN 11 — Tested supernatant – negative. Centrifuged. To S. ppt in water. 1.5 lts. 1½ alcohol. Centrifuging was not necessary.

4 — Precipitated with 1½ alcohol and let set in ice-box over week-end. Tested – negative. Most of supernatant was decanted.

JUN 14 — The remaining supernatant was poured-off. Sediment was centrifuged in cold. Then ppt was taken up in about

HOAc — 700 cc equally divided in 3 x 250 cc centri. bots. and then ppt. with 30 cc of Glacial acetic. Left in ice-box over-night.

JUN 15 — Centrifuged 3 bottles; poured-off supernatant; Took precipitate up in 700 cc of water and then neutralized with 10% Na OH. This then was precipitated with 1400 cc of alcohol

5 — after dissolving in it 30 gms of sodium acetate. Set in ice-box

JUN 16 — Poured as much of the supernatant as possible. Centrifuged the precipitate. Took ppt. up in 600 cc of water. ~~and dis~~

HOAc — Centrifuged in 3 x 250 bottles. Poured same in 3 other bottles. Put under stirrer and precipitated with 8 cc of glacial acetic acid. ~~Set~~ in ice-box

JUN 17 — The 3 bottles with S. ppt. was centrifuged in cold. Supernatant decanted and bottom layer taken up in

6 — water and then neutralized with 10% Na OH. This was poured in 1½ lts beaker; added 25 gms of sodium acetate and then ppt. with 1400 cc of alcohol. Set in ice-box overnight.

JUN 18 — Centfgd., taken up in H2O to vol. 500 cc., combined w. 1" portion S 58, acidf. neutrd. w. little HOAc, 150 cc. alc. added, set on ice over summer.

S S S 58

JAN 10 — Wt. bot (A) + subst. ltd. in $CHCl_3$ vapor in vac 8.7718 8.7706 (?)

8.7526 8.7479 8.7455 8.7424 8.7416
8.7409
8.4256
.3153

ASH

Wt. bot. + subst	8.4256	Wt. dish + ash	17.1693
" "	8.3299	" "	17.1693
	.0957		

Ash 0.0%

JAN 14 Titration

Subst wet w. few cc. H_2O, diss. in 10.00 cc. N/14 HCl (x1.003) & titrd. back w. N/14 (x1.038) NaOH To complete soly 10.00 10.38

25.20
20.00
5.2 5.20 5.40
15.78
HCl 10.03
0.411 cc. N ← 5.75

Titr. further, to bit. blue w. thymolphth. (cap$_H$ 10)

767

29.40
20.00
9.40
.15
9.25

Indic. bl. 25.53
25.38
.15

9.60
.15
9.45
5.75
15.20 = 1.086 cc. N

Total ~~289~~ 290

JAN 21 — Made up to 50 cc., centfgd. from traces insol.

OPTICAL ROTATION

l = 1 Bl = −0.06 .05 .06 Rdgs: +1.85 1.87 1.86

$$[\alpha]_D = + \frac{192}{.3153 \times 2} = +304.5°$$

$[\alpha]_D$ +304.5°

MICROKJELDAHLS 3 cc. samples

cc. N/70 HCl (x0.986)	10.00	9.86	10.00
" " NaOH (x0.996)	4.96	4.96	5.00
		4.90	
		.1	

In sample .01892 g.

4.80 × 0.2 = 0.96 mg N

N 5.1%

SSS 59 I

	6/4	Abbe I	Dx	V-21, 25, 28	3, 3, 14	6/5	6/7
	6/7	"	"	V-21, 28	14, 1	6/8	6/9
	6/18	"	"	V-11, 21, 25, VI-1	3, 7, 2, 3	6/10	6/11
	6/10	"	"	VI-11, V-1	2, 10	6/11	6/12
dense	6/11	"	"	VI-1	15	6/12	6/14
	6/16	"	"	VI-1, V-28	4, 8	6/17	6/19
	6/17	"	"	VI-9	16 (105)	6/18	6/19
	6/18	"	"	VI-9	15	6/19	6/21
	6/21	"	"	VI-9	12	6/22	6/23
	6/22	"	"	VI-9	12	6/23	6/24
	6/23	"	"	VI-9, 14	5/10	6/24	6/25
	6/24	"	"	VI-14	12	6/25	6/26
	6/25	"	"	VI-14	12 (173)	6/28	6/29
	7/14	"	"	VI-30	12	7/15	7/16
	7/15	"	"	VI-30, VII-7	18	7/16	7/17
	7/16	"	"	VII-7	12	7/17	7/19
	7/21	"	"	VII-7	12	7/22	7/23

FLASKS 237
LITERS 355.5

355.5 liters

9/27/26 All above lots were ppt. with 1.3 alc. with excep. dense 1.4
Total S. solution after dissolving of crystals & rinsing was 4.500 cc. This was precipitated with 1.000 cc of N/50 HCl and then put in ice-box over lunch hour. Clear top solution was siphoned-off & rest centrifuged in 8 bottles at ten minute interval. Clear solution was poured in 14 l. jar & each precipitated was washed with 50 cc of N/50 HCl and poured into major lot.
Total S. solution 5.000 cc, ppt. with 8.000 cc alc
Let stand overnight

2

9/28 Supernatant tested - negative then siphoned-off sediment poured into 8 bot. Centrifuged

Supernatant poured off & sediment taken up in 4 bottles. These were neutralized with 10% NaOH. Then centrifuged.
9/23/26 Clear solution was poured in 4 L. battery jars and ppt. taken up in 50 cc H_2O for each bottle. Centrifuged & poured into jar. Total S. solution 1000 cc + 50 gms acetate + 600 cc alcohol, all cooled. Then let stand in ice-box overnight.
3

10/2/26 The whole was poured into 8 bottles & centrifuged in cold. Supernatant thrown away.

10/4 Sediment in the 8 bottles were taken up in 125 cc of H_2O that is each bottle and then 4 cc of glacial acetic was added to each. This sol. was let to stand overnight in the ice-box.
HOAc

10/5 Centrifuged. Poured supernatant in a beaker to be tested — positive - added 300 cc alcohol. Centrifuged. Precipitates of ea. of the 8 bot. were dissolved in about 60 cc of H_2O & neutralized with 10% NaOH. Then poured into a 2 lt. beaker. Added 75 gm. of sodium acetate. Total S 700 cc Alcohol 400. Let stand overnight
4

10/6 Tested — positive. 100 cc alcohol added. Tested — negative. Centrifuged. Poured away supernatant.

10/7 Dissolved in about 600 cc of H_2O. Centrifuged. 1 cc. of glacial acetic added to each bottle (3). Let stand overnight.
HOAc

10/8 Centrifuged. Taken up in water (200 cc in ea. bot (3)) and neutralized with 10% NaOH.
5

10/9 ~~Centrifuged~~. Total S 600 cc — alcohol 300 cc — 25 gms sodium acetate. Set stand in ice-box

10/11 Test — negative. Centrifuged in cold.

OCT 14 1926 — Ppts. taken up in H_2O until, at vol. of ca. 700 cc. all but traces in solns. Made acid to Congo red w. HCl, centfgd. Grayish ppt. washed w. dil. HCl, centfgd., supnt. added to next lot. ~~Supnt.~~ Main supnt. neutrd. to Congo w. NaOH, few drops HOAc added. OCT 15 — Centfgd. Supnt —, colored. Discarded. Ppt. taken up in H_2O to ca. same vol. as before, acidified w. 2:1 HCl, centfgd. Run back w. NaOH as before, but nec. to add a little alc. before all S ppted. Taken up in H_2O, neutrd. to litmus w. NaOH, let stand over night w. saliva, since supnts.

SSS 59 Type I

				Flasks	Ppt.	Stocked
9/27/26	Abe I	Dx	IX - 8	12	9/29	9/30
9/28	"	"	"	12		
9/29	"	"	"	12	10/1	10/2
9/30	"	"	"	12		
10/1	"	"	"	12	10/2	10/4
10/4	"	"	"	12	10/5	10/6
10/13	"	"	VI - 30	12	10/14	10/15

126 liters

Flasks	84
Liters	126

All above batches were precipitated with 95% alcohol

OCT 18 Acidified until congo paper turned blue. Let stand in ice box over lunch hour. Centrifuged in 4 bottles. Poured clear supernatant in 6 L jar. Washed ppt with 100 cc N/10 HCl. Centrifuged & then added to main lot. Total S 1400 cc Alcohol 2200 cc

OCT 19 Tested - negative. Centrifuged in cold. Acid ppt washed 2 times & centrifuged in cold. Bottom layers were dissolved with 10% NaOH. Let stand overnight

OCT 20 Centrifuged. Finding top solution negative whole lot was stirred up & precipitated with Alcohol. Total 4 500 cc - Alcohol 500 cc

OCT 21 Centrifuged, dissolved in water, then centrifuged. Volume Taken in about 350 cc H_2O & Precipitated with 10 cc acetic acid. Let stand overnight in ice box

OCT 27 Clear supernatant poured off into beaker, about 400 cc & added to this 200 cc Alcohol.

Oct. 28 Precipitate taken up in 350 cc H_2O neutralized with 10% Na_2CO_3. Poured into beaker 10 gms $NaC_2H_3O_2$ added & then ppt. with 150 cc alcohol. Centrifuged in cold. Poured off supernatant & dissolved ppt. in about 600 cc H_2O To this was added saliva and let stand overnight.

Oct. 29 Centrifuged in cold. Supernatant about 400 cc was ppt. with 20 cc 10% ~~magnesium~~ alcohol & centri. ~~Ppt was taken up~~ Then again was ppt. with 130 cc of Alcohol.

OCT 30 Supernatant poured off + precipitate taken up in 300 cc H_2O. Then acidified with 9 cc Acetic acid glacial. Left stand in ice-box over week end.

NOV 1 – ~~Centfgd.~~ Supnt. treated with just enough alc. for ppt. to form. Ppts. from both fractions diss. in H_2O + HCl, centfgd., run back w. 10% NaOH until Congo no longer blue.

NOV 2 – ~~Centfgd.~~ Supnts. (A, B) ~~treated~~ w. sm. amts. alc. until all S ppt'd. NOV 3 – ~~Centfgd.~~ Main fracts. diss. in H_2O + few drops 2:1 HCl, centfgd. from sm. amt. ppt., run back w. 10% alk. until faintly just acid to Congo. Process repeated, incl. saliva treatment, until white product obtd, at same stage as main portion. Combined with this. Not very much.

Total 481.5 ?

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contd. from p. 2

had shown ~~traces~~ glycogen. NaOAc added & ppt'd. w. ½ vol. chilled alc. Taken up in H_2O, ~~acidif. to Congo w. HCl, neutrd. back as before, centfgd, ppt. taken up in H_2O.~~ NOV 9 – Combined ppts. susp'd. in ca. 500 cc. H_2O, acidif. w. 1:1 HCl until max. amt. in soln. Centfgd. NOV 10 – Run through 8 in Berkefeld V previously washed w. N/10 HCl. NOV 11 – Washed through w. 100 cc. N/50 HCl. Soln. still opalescent, so run through acid-washed 8" Berkefeld W. NOV 12 – 13 Filtrate and washings neutrd. w. 10% aq. NaOH until 1st considerable permanent ppt. (59A) Centfgd. Supnt. neutrd. further to max. pptn. (59B) NOV 15 – 7/6 Centfgd. Supnt. –, discarded. A & B ea. taken up in H_2O + little 1:1 HCl & chilled redistd. alc. added. A centfgd, then more HCl (2:1) added to ea. supnt., A ppt'g. quant., B giving active supnt. on centfgn. This (C) treated with more alc. Very little ppt. discarded. A rediss. in H_2O, ca. 20 cc. 1:1 HCl added, & at vol. of ca. 100 cc. ppt'd. w ca 3 vol. chilled

S S S 59

redistd. alc. After ½ hr. centfgd. (B) reppt'd. similarly. A & B redis. as well as possible & reppt'd. in same way. Taken up in H_2O. NOV 17-
Dis. as well as possible to thick paste w. ca 75 cc. H_2O, reppt'd. w. redistd. alc. without adding more HCl. Washings repeated w. H_2O 1" until product mainly misol., finishing each time w. redistd. alc. Washed 2x w. H_2O alone, then alc. alone, then again w. H_2O, adding alc. just before centfg. Washings w. H_2O & alc. repeated until both ppt. & washings Cl- free, then washed w. redistd. alc. redistd. acetone & filtered. Dried. Slightly discolored because allowed to get too hot.

A : 2.45 g.
B : 5.1 g.

Ppt in. Titer

A 30 cc. meas. in vol. flask, 0.8 cc. removed, made up to 50 cc. 1:500 soln.

B 35 cc. meas as above 3.5 cc. removed, made up to 50 cc. 1:500 soln.

Both fractions + 1:8,000,000.

S S S 59 A

DEC 10 — Wt. bot (E) + subst 7.5296 7.5290 7.5284 7.5284
Made up to 50 cc. 7.3571
.1713
.0002
.1711

ASH

Wt. bot + subst 7.3571
" bot 7.2712
.0859

Wt. dish + ash 17.1700
" " 17.1698
$CaSO_4$ .0002
" Ca .00006

Ash 0.1%

ACID EQUIVALENT

DEC 20 Subst moistened w. H_2O, diss. in 10 cc. N/14 HCl (×1.003) & titrated back to disappearance of isoelec. subst. w. N/14 NaOH (×1.038). Soln. still acid to litmus.

cc. NaOH 34.03 13.23
21.29 10.03
12.74 3.20 cc. N/14 NaOH excess, or 0.2286 cc. N NaOH
0.1711 g. req.

ACID EQUIV. 749

OPTICAL ROTATION

$l = 2$ Bl. −0.04 −.03 −.02 Rdgs. +2.05 2.05

$$[\alpha]_D = \frac{2.08}{2 \times 2 \times .1711} = +304°$$

$[\alpha]_D$ +304°

ACID EQUIV. ON A & B MIXED

Wt. bot + subst 7.2712
" " 7.2480
" A .0232

Wt. bot + subst 7.2866
" " 7.2353
" B .0513
" A .0232
.0745

Diss. in H_2O + 3 cc. N/14 HCl (×1.003). To disapp. of ppt. Titr. back w. N/50 NaOH (×1.049). then to 1° red w. phenol red.

cc. NaOH 38.72 40.99
24.15 38.72
14.57 2.27 × 1.049 = 2.38 cc. N/50
× 1.049 15.29 or .0476 cc. N
10.72
$3.003 \times \frac{50}{14}$ 4.57 cc. N/50 or 0.0914 cc. N
0.0744 subst.

814 **1563**

4.57
2.38
6.95 cc. N/50 or .1390 N

535

MICRO KJELDAHLS 5 cc.

cc. N/70 HCl (×.986) 10.00 9.86 10.00
" " NaOH (.996) 5.50 5.48 5.50
4.38
4.28 × 0.2 = 0.856 mg N

N 5.0%

SSS 59B

DEC 10 Wt. bot (8) + subst 7.5281 7.5271 7.5269

Diss. as usual, made up to 31.8 cc.

Evapd. sl., to made up to 50 cc, centfgd. from traces sediment

	7.5269
	7.3676
	.1593

.1593	
.0003	
.1590	Ash-free.

ASH

Wt. bot + subst	7.3676	Wt. dish + ash	17.1698		Ash 0.19%
" "	7.2866	" "	17.1693		
	.0810	" $CaSO_4$	.0005	(×.29435)	
		" Ca	.00015		

OPTICAL ROTATION

$l = 2$ Bl. = −0.05 .02 .04 ✓ Rdgs. : +1.86° 1.88

$$[\alpha]_D = \frac{1.91 \times}{2 \times 2 \times .1590} = +300.3°$$

$[\alpha]_D$ +300.3°

MICROKJELDAHLS 5 cc. soln.

cc. N/70 HCl (×.986)	10.00	9.86	10.00	
" " NaOH (×.996)	5.94	5.88	5.83	
		3.98		N = 4.9%
		.1		
		3.88 × 0.2 = 0.776 mg. N		

N 4.9%

ACID EQUIVALENT

DEC 21 Wt. bot (E) + subst 7.6551 7.6539 7.6535

Diss. in 10.00 cc. N/14 HCl (×1.003).

			7.6535	
			7.2479	
			.4056	
NaOH (×1.038)	10.00	10.38	.0008	
	26.50	6.96	.4048	Ash-free
	19.80	17.34		
	6.70	10.03		
		7.31 ←	0.522 N cc.	

Titr. to disapp. of ppt.

775

Titr. to bright pink w. phenolphthalein

36.10	
26.53	
9.57	
9.94	
7.31	
17.25	1.232 cc N

328 total

PN

NUCLEOPROTEIN 101

OCT 29 1926 — Supnt. from 1st NP ppt'n. 10 l. PN IR, obtd. 10/28, filtered through double layer folded filter to remove susped. particles, & at vol. of 1 l., concd. in vac. to 125 cc. at low temp. OCT 30 — Centfgd. Ppt (A), sm. amt, washed w. H_2O contg. few drops HOAc. Main supnt. made faintly alk. w. N NaOH, giving ppt. (B). NOV 1, ~~ff~~ increasing or making definitely alk. Centfgd., supnt. neutrd. again to litmus (C). (B) washed w. H_2O. C treated w. = vol. Satd. $(NH_4)_2SO_4$. NOV 3 ~~rff~~ Ppt. (D) centfgd. off & diss. in a little H_2O. Only faint turb. on b., ~~ppt~~ on pouring into H_2O, as does not behave like typical globulin. Biuret. Supnt. (E) sátd. w. $(NH_4)_2SO_4$ giving further ppt. Centfgd., taken up in H_2O, neutrd., centfgd.

B morganic, probably $Mg(OH)_2$ or $Al(OH)_3$.

NOV 8 — E soln. resatd. w. $(NH_4)_2SO_4$.

NOV 10 — Centfgd., ppt. taken up in H_2O. Completely sol. Sm. amt. coag. on b. Soln. gives Millon test, violet biuret, definite, but not very strong Xanthoproteic test. Slight reaction for IS.

S. S. S. and Rare Earth Salts

NOV 6 - 1:200 solns. Na salts S 1, 2, 3, E.S.S. set up. ~~agst~~. 1 drop rare earth soln. added.

Salt Tested	Pn I	Pn III	Pn II	Frdl. B	Frdl. A
	Sol. Sp. Subst.				
6% Rb Cl	–	–	–	–	–
2% Cs Cl	–	–	–	–	–
Tl Cl (sata)	–	–	≠	≠	–
4% Ce NH_4 nitrate	++++	++++	faint opalesc.	–	–
2% Th "	++++	++++	++++	++++	++++
" La Cl_3	++++	++++	–	–	–
0.4% Nd nitrate	++++	++++	faint opalesc.	+	±
"(?) Pr "	++++	++++	" "	+	±
2% Er "	++++	++++	++	–	faint opalesc.
" Y "	++++	++++	++	–	" "
~~Cs~~ chloride	–	–	–	–	–

G. A. (56 C) ppts. only w. Th.

SS 61 I

						Precip.	Stocks
10/5/26	1/193/4	Dx	I X - 28	12		10/7	10/8
10/6	"	"	"	16			
10/7	"	"	"	12		10/8	10/9
10/8	"	"	"	16		10/11	10/13
10/11	"	"	"	12		10/13	10/14
10/13	1/196/4, 5	"	X - 5, 6	4, 7		10/15	10/18
10/14	1/196/4, 5	"	I X - 28, X - 5, 6	3, 4, 10			
10/15	1/196/5		X - 6	17		10/16	10/18
10/18	I/196/4, 5		X - 5, 6	4/8		10/19	10/20.
10/21	I/196/6		X - 15	12		10/25	10/26
10/22	"		"	12			
10/25	"		"	12	161	10/26	10/27
10/26	"		"	12		10/27	10/29
10/27	"		"	12	185	10/29	11/1
11/3	"		"	12		11/4	11/5
11/4	I/196/4		X - 20	12		11/5	11/8
11/8	1/196/4		X - 26	15		11/9	11/10
11/9	"		"	15		11/10	11/11
				Flasks 239			
				358.5			

All of above batches were precipitated with 1.3 of alcohol excepting last four which were precipitated with 1.4. This helped the centrifuging.

NOV 11

Total S. solution 5.5 l. added enough 1:1 HCl to turn Congo red, blue. Set in ice-box over lunch hour. Centrifuged. Poured clear supernatant in 15 l. jar. Precipitate was washed once with $\frac{N}{50}$ HCl. Centrifuged & poured into major lot.
Total S. solution 6000 cc Alcohol 9.600 cc
Set stand overnight

NOV 12

Tested - negative. Supernatant was poured into another 15 l. jar and closely neutralized it. This gave off a ht.

Major precipitate was centrifuged in 4 bottles. ~~Some~~ Second precipitate mostly crystalline was centrifuge. Both were taken-up in water & neutralized with 10% Alkali. Total S. 900 cc Alcohol 450

11-15 Centrifuged in 6 bottles. Tested - negative. Taken-up in water. Centrifuged. Poured into jar; added 50 gms Sodium acetate. Total S solution 1000 cc Alcohol 600 cc.

11-16 Some was centrifuged; tested - negative. Centrifuged; taken-up in water, about 900 cc. Precipitated with 35 cc Glacial acetic Let stand in ice box overnight.

11-17 Tested - positive; added 250 cc alcohol. Tested - negative. Centrifuged in cold; taken-up in water (700 cc) neutralized with 10% NaOH Total S. solution about 750 cc ~~Alcohol 275 cc~~ added 10 mouthfuls of sp.

11-18 Centrifuged. Dissolved bottom layer. Total S solution 800 cc & 30 gms sodium acetate & 400 cc alcohol. Let stand in ice-box over lunch hour. Tested - negative

11-20 Total S. solution 800 cc, added 25 cc Glacial acetic Centrifuged - poured-off supernatant. Let stand in ice-box

DEC 29 - Taken up in H_2O, diss. w. 1:1 HCl centfgd. DEC 30 ~~ff.~~ - Centfgd. again, supnt. pptd. as usual w. NaOH. After several hrs. in cold, centfgd., ppt. stirred & centfgd. again, ~~ppt~~ supnt. discarded.

JAN 3 1927 ff. - Diss. again in cold w. H_2O & HCl, centfgd. as before, & JAN 4 ~~run~~ through acid-washed Berkefeld V candle (8 in), then ~~through~~ W candle. Acidified very strongly w. 2:1 HCl & pptd. w. chilled alc. Ppt. taken up in H_2O & pptd. isoelec. 2" from acid side, then diss. in dil. acid, & at vol. of ca 500 cc. HCl added until remained clear on adding 2 vols. chilled redistd. alc. On adding xs 2:1 HCl, S.HCl pptd. Centfgd., rediss., acidif. strongly w. l. xs. 2:1 HCl & pptd. (at vol. of ca. 500 cc.) w. = vol. chilled redistd. alc. Centfgd. Taken up in ~~little~~ H_2O to thick mucilage, pptd. w. redistd. alc. JAN 14 - Centfgd., process repeated. JAN 17 - Soln. still ~~quite~~ appreciably brownish, so pptd. w. $Ba(OH)_2$ & worked up as was (58).

Type I -S- Oxidation.

(Total: 3.5 g.).

NOV 23 1926 — 0.55 g. 38A, 1.08 g. 43B, 1.08 g. 44A, 0.57 g. 41 added to 35 cc. 50% (by vol.) HNO_3 and viscous mixt. stirred until all but few particles in soln. Let stand at room temp. over night, then at 34-38° for ca 7 hrs. daily. After 3 days still unchanged S present, none after 5 days. Considerable mucic acid had sepd., so stirred & set in ice-box. DEC 1 — Crysts. centfgd. off & washed w. cc. 50% HNO_3 & washings added to main supnt. which was warmed as before. Crysts., washed w. 2 portions alc. & Et_2O = 0.4 gm. 212-8° (decompn). DEC 21 — More crysts. had sepd., so centfgd. off, washed w. sm. portion H_2O, then alc., Et_2O. = 0.55 g. M.p. decompn. 212-6° Supnts. rapidly evapd. on H_2O bath on 2 watch glasses w. stirring, evapd. 1st more w. few cc. 1:1 HNO_3, then H_2O, diss. in hot H_2O, chilled, & at vol. of ca. 15 cc. seeded w. mucic acid. After several days in ice-box centfgd. & ppt. washed w. little H_2O. 0.1 g. m.p. 213-7° (decompn). Soln. concd. to sm. bulk in vac., taken up in redistd. alc., distd. again in vac., taken up in abs. alc., repeated, again taken up in abs. alc. Let stand in ice-box protected from moisture. JAN 20 — Supnt. decanted from sm. crystn. residue & this washed w. little abs. alc. & filtered off. m.p. decompn. 195-205° w. prelim soft. & dark. Supnt. + ca 3 vols. dry Et_2O gave sm. amt. ppt., probably mucic acid which was centfgd. off. Strongly acid, but gave odor of NH_3 or $MeNH_2$ when made alk. & boiled. Probably partly mucic acid, partly NH_4NO_3. Supnt., with several more vols. Et_2O, gave heavy ppt., but on filtering this yielded a very small, hygroscopic residue on the paper. Dried in vac. over $CaCl_2$: 0.14 g. Acid to Congo, gives naphtoresorcin test, reduces weakly.

JAN 21 — Et_2O filtrate let stand in ice-box, but only deposited trace more amorphous material. Filtered off, filt. concd. to sm. bulk on H_2O bath, taken up in ca 10 cc. H_2O, centfgd. from trace insol. Few drops soln. give strong redn. w. Fehling, just as in case of oxn. w. Type III S. JAN 22 — Soln. treated w. 100 cc. N/10 I_2-BaI_2 (50 g. latter per l.) & 275 cc. (excess over usual 200 to neutr. sugar acid) N/10 $Ba(OH)_2$ (15.7 g. per l.) dropped in, w. stirring during 5 min. After 15 min. more centfgd. from insol. flocks (A) & 5 N H_2SO_4 added in sl. xs., then 20-30 g. C.A.F.K. $PbCO_3$ I & stirred vigorously until pale blue to Congo. Centfgd. Supnt (B) concd. in vac. to ca 50 cc. & let stand in ice-box. A shows α_D +.25° after removal of Ba quant. w. H_2SO_4, indicating that main fract. present in it. (B) yielded very little material appar. chiefly oxalic acid. Discarded. A soln. concd. dry in vac., taken up in a little abs. alc.

Type I S – Oxidation

OCT 20 1927 – Soln. rinsed into weighed dish w. a little redistd. alc. & evapd. in vac at room temp., finally warming & using oil pump to make product friable. OCT 28 – Yield: 0.29 g. Portion added to HOAc, mainly very soly, exc. for sm. amt. white gelatinous material.

Lemon Pectin

S S S 65

JAN 4 1927 — 1:1000 soln. lemon pectin made up, & from this, 1:10,000. Latter —, but former gave heavier turbidity in II antiserum than in others. JAN 5 — Ppt. had not settled, but became scaly, not jelly-like on centfg. 5 g. of pectin, disad. to a smooth paste the day before in 50 cc. 1:1 HCl, had set to a soapy cake. Ground up, pectic acid centfgd off. Supnt. + several vols. alc. gave sm. amt. ppt., which rediss. in saline & neutrd. gave very heavy, typical S ppt. w. II serum, no definite ppt. w. normal horse serum or I serum.

100 g. pectin ∴ diss. in N HCl (1 l.) & let stand over night. JAN 6 — Still clear paste, so ground smooth w. = vol chilled 2:1 HCl. JAN 7 — Soapy cake ground up w. more HCl (1:1) to render it sufficiently fluid, centfgd. in cold. JAN 8 — At vol. of 1.5 l., clear soln., ppt'd. w. 4 l. chilled alc. JAN 10 — Supnt. decanted, rest centfgd., ppt. taken up in ca 50 cc. H_2O, centfgd. from sm. amt. insol. matter & this washed w. little H_2O. 10 cc. 1:1 HCl added & subst. ppt'd. w. chilled redistd. alc., w. stirring. After several hrs. centfgd, supnt — w. II serum. Ppt. rediss. in H_2O, centfgd. from traces insol. matter, & at vol. of 30 cc. reppt'd. w. chilled redistd. alc. JAN 11 — Excess alc. added, since portion not ppt'd. was sp. Centfgd. repeatedly from increased amt. insol. matter. JAN 12 — This was amphoteric, gave viscous aq. soln., but was relatively inactive. Main soln., at vol. of 30 cc. gave milky soln. w. 6 vols. chilled redistd. alc., not improved by addn. of acetone. Few cc. HCl added. Nec. to add few drops HCl. Centfgd. Taken up in H_2O, centfgd to remove bone-blacked turb. still remaining. Filtered through Berkefeld, 2:1 HCl added, reppt'd. w. chilled alc. Centfgd, taken up in a little H_2O, reppt'd. w. acetone. JAN 15 — Taken up in ca. 10 cc. H_2O, incl. washings, stirred into 200 cc. redistd. acetone. Yield: 0.9 g.

This reacted w I serum !!?!

65A

JAN 13 — 45 g. pectin ground up to smooth paste w. 250 cc. 1:1 HCl. JAN 14 — Ground up in a mortar w. H_2O, centfgd; ppt. squeezed out in muslin, supnt. filtered through cotton plug, & at vol. of ca 200 cc., ppt'd. w. ca 800 cc. chilled alc. JAN 15 — Taken up in 20 cc. H_2O, (no I's in ppt) centfgd., 5 cc. 1:1 HCl added, ppt'd. w. sm. redistd. alc. JAN 17 — Taken up in ca 25 cc. H_2O. Soln. almost clear, but something sepd. on standing in cold ca 2 hrs. Centfgd. in cold. Sm. amt., — w. I serum, readily sol. in H_2O. JAN 18 — Supnt. reppt'd. as before, taken up in H_2O. JAN 19 — Centfgd., ppt'd. w. acetone, washed w. this. Yield: 0.5 g.

This time amphoteric residue, +++ w. I serum, obtained

SSS 65 B

JAN 31 Aq. supnt. from this residue, consisting of least sol. portion of the sp. gum, ppt'd. w. acetone centfgd! Repptd 2 X from H_2O in pres. of HCl w. redistd. alc., taken up in a little H_2O, centfgd. from dusty, ppt'd. w. redistd. acetone. Yield:

S S S 65

Wt. bot ( ) + subst (100° in vac) 7.5870 7.5861
7.2785
.3076

Diss. in H_2O, titrated, made up to 30.76 cc.

ASH

Wt. bot + subst 7.2785
" " 7.2349
.0436

Wt. dish + ash 21.9185 ✓
" " 21.9187

Ash-free

Acid Equiv.

cc. N/14 NaOH (X1.038) to pink w. phthl. 10.25
10.64 = 0.76 cc. N

Acid Equiv. 405

OPTICAL ROTATION

$l = 2$ Bl. = −0.02 −.02 Rdgs. +2.08 2.10 2.12 2.10

$$[\alpha]_D = + \frac{212}{2} = +106°$$

$[\alpha]_D$ +106°

MICROKJELDAHLS 3 cc.

cc. N/70 HCl (X.986) 10.00 9.86 10.00
" " NaOH (X.996) 9.65 9.61 9.65
.25
.1
.15 X .2 = 0.03 mg. N in 30 mg.

N 0.1%

HYDROLYSIS

Ppts. w. Cu^{++}, neutr. & basic Pb^{++}, $(UO_2)(NO_3)_2$, not w. cold satd $Ba(OH)_2$.
Biuret —.

S S S 65A

Wt. Bot (M/ + subst. ($CHCl_3$, in vac) 8.2388 8.2384
~~8.2380~~ 7.8746
.3638
.0006
.3632

Dis. in H_2O, made up to 36.32cc.

JAN 31

ASH

Wt. Bot + subst	7.8746	Wt. dish + ash	17.1663
" "	7.8176	" "	17.1660
	.0570	" ($CaSO_4$)	.0003
		" Ca	.0001

Ash 0.2%

OPTICAL ROTATION

$l = 2$ Bl. = -0.05 -.05 Rdgs. 2.27 .30 .29 ✓

$$[\alpha]_D = \frac{234}{2} = +117°$$

$[\alpha]_D$ +117°

ACID EQUIVALENT 10cc.

cc. N/50 NaOH ~~to~~ pink w. phthl. (x1.049).

12.2 12.80 = .256c. N

.100 g.

Acid. Equiv 390

MICRO KJELDAHLS

Ppts. in 1:200 (satd.) soln. w. Cu^{++}, $UO_2(NO_3)_2$, & $Ba(OH)_2$ soln.

Biuret –, after transitory yellow color (1:100 soln.).

Lemon Pectin Antigen

JAN 11 1927 — 2 g. moist inner white skin of fresh lemon shaken in bottle w. glass beads 1 hrs. w. 19 cc. saline + 1 cc. 5% $PhOH$. Little disintegration.

Better results on grinding 2 g. with quartz sand, making up to 100 cc. w. saline, neutlg. to Litmus (color change) and decanting from coarser particles.

DEC 6

G.A.

1.0787 gm. of the partly decomposed Fraction I (dried in vacuo over boiling Toluene) was dissolved in 10 cc 1-1 HNO_3 and heated on the water bath. No mucic acid formed.

Willstatter Schudel on Fraction I dried in vacuo over $CHCl_3$-CCl_4-H_2O.

.141 grams Ca salt. 20 cc I .0987N = 8.34 cc N/10 Iodine.
11.53 cc $Na_2S_2O_3$ .0988N.

$\frac{187.5}{x} = \frac{10}{8.34}$ $x = 156.4$ $\frac{156.4}{141} = \frac{110.8}{10}$

Sugar content equivalent to 110.8% Calcium Aldobionate.
Walter Goebel.
Jan. 8, 1928.

Sugar-from Acetone mother liquors of fraction 3. Crystals formed slowly upon standing in the icebox. Shape- Hexagonal prisms and rosettes. Recrystallized from MeOH. M.P. 160-168 - 0.1gm dissolved with 1 cc 1-1 HNO_3. White crystals of mucic acid. M.P. 206-7 uncor. Slow heating. Sugar must be Galactose.
Willstatter-Schudel.

Fraction 2 - 121% Calcium aldobionate
144.5 Mg sample 144.5 × 1.21 = 174.8
= 174.8 Mg Calcium aldobionate as Glucose
$\frac{187.5}{174.8} = \frac{10}{x}$ $x = 9.32$
~~18~~ $\frac{90}{x} = \frac{10}{9.32}$ $\frac{187.5}{174.8} = \frac{90}{x}$ $x = 83.9$ $83.9/145 \times 100 = 58.0$ as Glucose

Fraction 3 128% Ca Aldobionate
145.2 mg sample $\times 128 = 185.8$
$\frac{187.5}{185.8} = \frac{90}{x}$ $x = 89.2$
$(89.2 \div 145.2) \times 100 = 61.45\%$ as Glucose

Sample II Aldose sugar Willstätter-Shüdel -
equi-to 120% Ca aldobionate

Sample III
128% Ca aldobionate -

S 66

MAY 23 1927 + ff — 49 g. lot prepd. like ~~62~~ 56 B+C, activity 1:4,500,000 b. 4 hrs. w. 57 cc. conc H_2SO_4 in 2 l. H_2O. Let cool, neutrd. w. 8× recrystd. $Ba(OH)_2$ (acid. overnutrd.), balanced w. H_2SO_4. Let stand in ice-box. Concd. to sm. bulk, again adjusted so that very faint xs. H_2SO_4, & w. $CaCO_3$ & bone-black, concd. to syrup, poured into xs. redistd. MeOH, set on ice. Few crysts. sepd. among otherwise amorphous mass, over summer.

OCT 10 1927 – After previously centfg., washing 2× w. redistd. MeOH, H_2O added until most of salt became pasty, supnt. poured off + evapd. to remove MeOH, returned, + aq. vol. of ca 60 cc. redistd. MeOH cautiously added until sl. permnt. turby. Warmed on H_2O bath to dissolve sepd. solid, chilled in ice-H_2O, centfgd. in cold. Supnt. decanted, ppt. dissd. in ca 30 cc. H_2O, reppted. as before. Chilled!

S.S. from Hemolytic Streptococcus "S3"

MAR 17 1925 – Supernatant from N.P. of Avery – extd. strep. from 6 l. culture, markedly acid w. HOAc, at vol. of ca 180 cc., concd. to 35 in vac., centfgd. from sm. amt. sepd. protein (biuret test) & pptd. w. 50 cc. alc. Let stand 0.25 hr. in ice-box, centfgd. in cold. MAR 18 – Ppt. inactive, gave nucleic acid test after b. w. HCl & adding Fehling soln. Supernatant concd. to ca. 5 cc., pptd. by diln. to 50 w. alc. Product which sepd quickly centfgd., leaving supernatant (A) to stand over night. Ppt. taken up in ca. 10 cc. H_2O, neutr. w. NaOH, centfgd. Supernatant inactive, + drop ~~in~~ N dil. NaOH + little saline (B) Soln. poured off. (C)

(B) reacts strongly w. S3 serum. Supernatant (D)

(C) slowly deposited ppt. & crystals. Centfgd. Ppt. taken up in a little H_2O, neutr., centfgd. Reacts well w. S3 serum.

(D) Concd. to dryness, taken up in a little H_2O, neutr. Inactive.